Reagent deposition for rapid multiplex pathogen identification in human blood culture samples

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Reagent deposition for rapid multiplex pathogen identification in human blood culture samples

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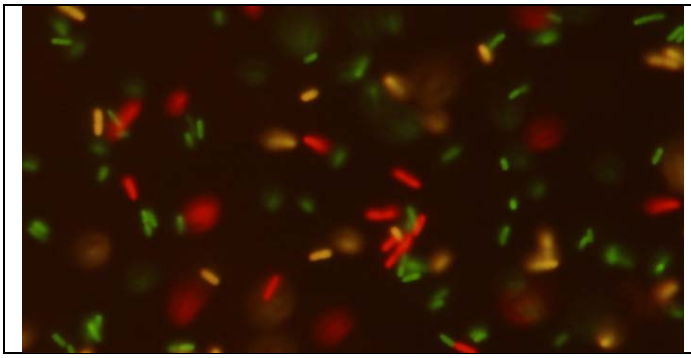
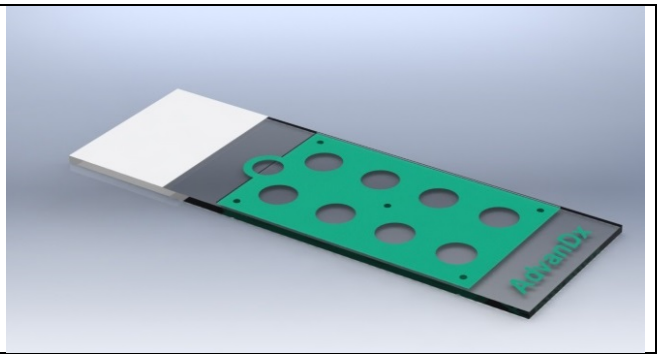
Blood stream infections led to 135,000 deads annually in EU and fast treatment significantly increases the survival rate. This condition is diagnosed by means of blood cultures (19 Mill blood cultures are drawn annually in EU). In this work, a multiplex peptide nucleic acid / fluorescence in-situ hybridization assay is used (PNA-FISH) for diagnosis of hospital acquired bacteria, such as staphylococcus aureus, enterococcus faecalis, E. coli, candida albicans etc. The test covers 90-95 % of the species by prevalence. It is based on a microscope slide format with inspection of the fluorescing bacteria viewed in a dual-color microscope configuration. The test takes 20-30 min to perform.

In order to lower the cost of the test, rapid automated reagent deposition is needed. Here, ultrasonic spray coating of polyvinyl alcohol/PNA-probes on microscope glass slides is presented. Different wetting regimes are explored in order to control the spot profile from convex to concave. The spray coated test are compared with manually prepared tests to ensure that the same performance is obtained.





Left) Example of imaging of pathogens in human blood culture samples using a 60x oil immersion objective. Green: E. Coli; Red: P. Aeruginosa; Yellow: K. Pneumonea. Right) Microscope slide used for the test. In each circular well, three pathogens can be detected.